REMARKS

By the present amendment, claims 1, 2 and 25-29 have been amended and claims 32-47 have been deleted as being drawn to a non-elected invention. The amendment renders claims 1-31 pending in the present application. The amendments to the claims have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicant reserves the right to pursue any of the deleted claims in a further continuation, continuation-in-part or divisional application. The amendment does not contain new matter and its entry is respectfully requested.

The Official Action dated March 27, 2002 has been carefully considered. It is believed that the amended claims submitted herewith and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

35 USC §112, Second Paragraph

The Examiner has objected to claims 1-31 under 35 USC §112, second paragraph as being indefinite. In particular, the Examiner comments that in claims 1, 2 and 29 it is unclear if the first and second media are materially different. In response, claims 1, 2 and 29 have been amended in order to specify that the second culture medium does not contain a T cell mitogen. Support for this amendment can be found on page 10, lines 19-20.

The Examiner has also objected to claims 2 and 25-28 under 35 USC §112, second paragraph as being indefinite. In particular, the Examiner comments that the term "XLCM" is a trade mark which should not be used in a claim. In response, claims 2 and 25-28 have been amended in order to define "XLCM" as "a conditioned medium prepared by stimulating umbilical cord blood cells with mezerein and concanavalin A". Support for this amendment can be found in the specification, for example on page 12, lines 8-10 and on page 17, lines 21-23.

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In view of the foregoing, we respectfully request that the objections to the claims under 35 USC §112, second paragraph, be withdrawn.

35 USC §102

The Examiner has raised various objections to the claims under 35 USC §102 as being anticipated by several references. Before discussing the cited references, it may be useful to summarize the present invention. The present invention relates to improved methods for preparing TcR$\gamma\delta^+$ T cells. In one aspect, the method involves culturing cells in a first culture medium comprising a T cell mitogen, interleukin-2 and interleukin-4 and then subsequently sub-culturing the cells in a second culture medium comprising interleukin-2 and interleukin-4 (claim 1). In another aspect, the method involves culturing cells in a first culture medium comprising the conditioned medium known as XLCM and then subsequently sub-culturing the cells in a second culture medium comprising interleukin-2 and interleukin-4 (claim 2). As can be seen in Examples 4 and 5 of the present application, the sub-culture in the second culture medium greatly enhances the yield of TcR$\gamma\delta^+$ T cells as without the sub-culture step, less than 5% of the cells are TcR$\gamma\delta^+$ (see page 20, line 29 and page 21, line 29). However, with the inclusion of the sub-culture step, the yield of TcR$\gamma\delta^+$ T cells rises to greater than 50% (see page 20, line 30 and page 21, line 30). Consequently, there are several advantages of the method of the present invention including: (1) increased yield of TcR$\gamma\delta^+$ T cells; (2) the removal of the T cell mitogen which makes the cells better suited for therapeutic uses; and (3) the method does not require initial fractionation or enrichment of the starting cells.

The Examiner has objected to claims 1-5 and 10-28 under 35 USC §102(b) as being anticipated by Bell et al. (WO 98/33891) and Bell et al. (U.S. Patent No. 6,194,207). We respectfully disagree with the Examiner for the reasons that follow.

We will discuss both of the Bell et al. references together as the disclosures of the two are identical. The Bell et al. references describe the expansion of TcR$\gamma\delta^+$ T cells in Example 8. In particular, Bell et al. cultures cells in a media comprising 5% conditioned

medium (XLCM) and 5% plasma. Importantly, Bell does **not** sub-culture the cells in a second culture medium comprising IL-2 and IL-4 (and not containing the CM). Using the one step method, Bell reports that the proportion of TcR$\gamma\delta^+$ T cells was about 18% in later stages of the culture which is significantly lower than the amount obtained using the improved method of the present invention.

In addition, Bell does not teach or suggest the method of claim 1 as Bell does not culture cells in a first culture medium comprising a T cell mitogen, IL-2 and IL-4 and then subsequently sub-culture cells in a second culture medium comprising IL-2 and IL-4. Contrary to the Examiner's assertion, the conditioned medium used in Bell does not contain significant amounts of IL-4 as indicated in Table 1.

In view of the foregoing, we respectfully request that the objections to the claims under 35 USC §102(b) be withdrawn.

The Examiner has objected to claims 1-5, 7-14, 16-23 and 25-28 under 35 USC §102(a) as being anticipated Skea et al. (J. of Hematotherapy & Stem Cell Res., 8:525-538, 1999) as well as Skea et al. (J. of Hematotherapy, 8:129-139, 1999). We respectfully disagree with the Examiner for the reasons that follow.

Neither of the Skea et al. references are citeable against the claims of the present application as both articles were published in August 1999, which is well after the filing date of November 4, 1998 for the priority application, serial no. 60/107,006. The claims of the present application are supported in the priority application. Example 3 of the priority application describes a method of expanding TcR$\gamma\delta^+$ T cells by culturing cells in a first culture medium comprising XLCM and then sub-culturing in a second culture medium comprising IL-2 and IL-4 (claim 2). Example 4 of the priority application describes method of expanding TcR$\gamma\delta^+$ T cells by culturing cells in a first culture medium comprising concanavalin A, IL-2 and IL-4 and then sub-culturing the cells in a second culture medium comprising IL-2 and IL-4 (claim 1). Consequently, the claims of

the present application are supported in the priority application and therefore neither Skea et al. reference is citeable under 35 USC §102(a).

Further, it is worth noting that even if the Skea et al. references were citeable under 35 USC §102(a), they would not be relevant as they do not disclose the subculture of the cells in the absence of the mitogen or XLCM.

In view of the foregoing, we respectfully request that the objections to the claims under 35 USC §102(a) be withdrawn.

35 USC §103

The Examiner has objected to claims 6-8 under 35 USC §103(a) as being unpatentable over Bell et al. (WO 98/33891) in view of Thomas et al. (U.S. Patent No. 5,877,299). We respectfully disagree with the Examiner for the reasons that follow.

As mentioned above, Bell in no way teaches or remotely discloses the improved methods of the present invention. The deficiencies in Bell are not remedied by Thomas et al. Thomas et al. is concerned with a negative selection method for isolating human hematopoietic progenitor and stem cells in a sample. Thomas et al. is not concerned with novel methods for preparing TcR$\gamma\delta^+$ T cells. Claims 6-8 under objection depend from claim 1 and therefore carry with them all of the novel and inventive features of claim 1 as previously described. One of skill in the art having read Bell and Thomas would in no way be motivated to develop an improved method for expanding TcR$\gamma\delta^+$ cells as is claimed in the present application.

In view of the foregoing, we respectfully request that the objections to the claims under 35 USC §103 be withdrawn.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

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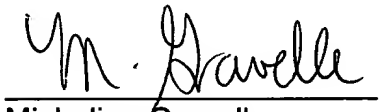
In view of the foregoing comments and amendments, we respectfully submit that the application is in order for allowance and early indication of that effect is respectfully requested. Should the Examiner deem it beneficial to discuss the application in greater detail, he is kindly requested to contact the undersigned by telephone at (416) 364-7311 at his convenience.

Respectfully submitted,

DAVID N. BELL ET AL.



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Version with markings to show changes made

In the Claims

Claims 1, 2 and 25-29 have been amended as follows:

1. (Amended) A method for expanding TcR$\gamma\delta^+$ T cells in a starting sample comprising:

(1) culturing cells in the starting sample in a first culture medium comprising (a) a T cell mitogen, (b) interleukin-2 and (c) interleukin-4; and

(2) culturing the cells obtained in step (1) in a second culture medium comprising (i) interleukin-2 and (ii) interleukin-4 to expand TcR$\gamma\delta^+$ T cells, wherein said second culture medium does not contain a T cell mitogen.

2. (Amended) A method for expanding TcR$\gamma\delta^+$ T cells in a starting sample comprising:

(1) culturing cells in the starting sample in a first culture medium comprising [XLCM] a conditioned medium prepared by stimulating umbilical cord blood cells with mezerein and concanavalin A; and

(2) culturing the cells obtained in step (1) in a second culture medium comprising (i) interleukin-2 and (ii) interleukin-4 to expand TcR$\gamma\delta$+ T cells, wherein said second culture medium does not contain a T cell mitogen.

25. (Amended) A method according to claim 2 wherein the [XLCM] conditioned medium is present in an amount from about 1 to about 25%.

26. (Amended) A method according to claim 2 wherein the [XLCM] conditioned medium is present in an amount from about 2 to about 20%.

27. (Amended) A method according to claim 2 wherein the [XLCM] conditioned medium is present in an amount from about 2.5 to about 10%.

28. (Amended) A method according to claim 2 wherein the [XLCM] conditioned medium is present in an amount from about 5%.

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29. (Amended) A method for obtaining TcR$\gamma\delta^+$ T cells from a sample from a patient with chronic myelogenous leukemia comprising:

(1) obtaining low density mononuclear cells (LDMNC) from the sample;

(2) depleting the cells obtained in step (1) of $CD33^+$ cells;

(3) culturing the cells obtained in step (2) in a first culture medium comprising (a) a T cell mitogen, (b) interleukin-2 and (c) interleukin-4; and

(4) culturing the cells obtained in step (3) in a second culture medium comprising (i) interleukin-2 and (ii) interleukin-4 to expand TcR$\gamma\delta^+$ T cells, wherein said second culture medium does not contain a T cell mitogen.

Claims 32-47 have been deleted.

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